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EFFECTS OF ACETYLSALICYLIC ACID IN STROKE PATIENTS EVIDENCE OF NONRESPONDERS IN A SUBPOPULATION OF TREATED PATIENTS

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ABSTRACT:

Platelet reactivity (PR) was tested two and 12 hours after acetylsalicylic acid (ASA) intake in 82 stroke patients, aged 59 ± 14 years (33 female and 49 male). 10% of these patients showed a pathologically enhanced PR at least two hours after intake of 500 mg ASA (= primary ASA-nonresponder (PNR)). Only 10 hours later, a further 26% of these ASA treated patients exhibited a pathological platelet reactivity (>1.25) (= secondary ASA-nonresponder (SNR)). Single ASA dosages of 500 mg or 200 mg were of identical effectiveness. Additional administration of metoclopramide in combination with 100 mg ASA was more effective as compared to a single dosage of 1000 mg ASA. Those who were SNR at onset of ASA therapy remained SNR as well 28 days later. The change from a normal, ASA corrected PR, to pathological PR values before a period of 12 hours ended seemed a sudden and irreversible event that could only be corrected by the next ASA application.

INTRODUCTION:

The efficacy of acetylsalicylic acid (ASA) in stroke prevention has been disputed up to now (1, 2, 3, 4, 5). A laboratory controlled administration of ASA did not prove any effectiveness of ASA in preventing stroke (6). Although it is accepted that the most important effect of ASA is mediated by the inhibition of the prostaglandin pathway in platelets (7, 8) it should be borne in mind that ticlopidine compared to ASA is more effective in stroke prophylaxis (9, 10). However ticlopidine does not influence the prostaglandin pathway in platelets (11). These findings raised the question as to whether it is wise to dismiss all the old well-known effects of ASA on platelets (12) and to postulate the prostaglandin pathway as the only

KEYWORDS: Platelet reactivity, stroke, acetylsalicylic acid dosage

way to explain the action of ASA on platelets (13). The platelet reactivity test - a platelet test that acts rather as a global test of platelet function - showed an impressive difference between healthy persons and patients suffering from cerebrovascular disease (CVD) such as transient ischemic attacks (14). It was thus aim of this study to describe the influence of different ASA dosages on the parameter platelet reactivity (PR) in patients suffering from CVD under ASA treatment. In addition the influence of different time periods after ASA intake on PR index values was of interest because the time lag between two consecutive ASA dosages ranges between 6 and 12 hours under clinical conditions.

PATIENTS:

82 stroke patients (33 female and 49 male, age 59 ± 14 years) were included in the study. All patients were inpatients of the University Hospital. All patients denied smoking. 31 of these patients were receiving antihypertensive treatment. 21 patients were diabetics. 12 patients showed abnormalities of the lipid metabolism. Hyperuricemia was seen in 3 patients. According to good clinical practice, 3 x 500 mg ASA were given routinely p.o. to all patients. So far a modification of dosage and dosage frequency was made, all patients gave informed consent to this experiment.

METHODS:

The platelet reactivity test, a further modification of the WU and HOAK test (15) described earlier (14, 16), was introduced to determine platelet function induced by the blood sampling procedure. In general, 300 µl blood were suspended in both EDTA and in EDTA-formaldehyde. Red blood cells were counted in both samples. The platelets activated and aggregated by blood collection were dissolved in EDTA and fixed in EDTA-formaldehyde. Centrifugation causes aggregates to sink to the bottom so that these are not present in the supernatant fluid while single platelets remain in the supernatant fluid. The platelet reactivity (PR) index increases in proportion to the number of activated (i.e. aggregated) platelets.

$$\frac{\text{Platelets in EDTA} * \text{Red Blood Cells in Formalin-EDTA}}{\text{Platelets in Formalin-EDTA} * \text{Red Blood Cells in EDTA}} = \text{PR}$$

This test system was standardized in 110 healthy persons (50 women and 60 men, age 48 ± 16 years). Mean of healthy ones was 0.98, standard deviation was 0.09. The normal range of the test system is mean ± 3SD i.e. below a PR index value of 1.25 (14). Blood samplings routinely were performed at 8.00 a.m. (12 hours value) or two hours later (2 hours value). Patients had their breakfast from 6.45 - 7.15 a.m., i.e. about one hour before blood sampling.

RESULTS:

a) Influence of different ASA dosage on PR after two hours

Before ASA intake and two hours after ASA application platelet reactivity values were determined in 20 patients TAB.1. Between the intake of two different ASA dosages ASA free intervals of 24 hours were observed in each

case. Patients without change of PR values under a given dosage of 100 mg ASA received an additional 100 mg ASA in combination with 10 mg metoclopramide. With a dosage of 50 mg ASA 30% of all patients did not reach the normal range of the PR test. With an ASA dosage of 100 mg 20%, with 200 mg or with 500 mg 10% and with 1000 mg 5% of all PR index values remained pathological. All patients whose platelets did not react after 100 mg ASA showed normal PR values after 100 mg ASA + 10 mg metoclopramide. Normalization of the PR index was seen even in patients whose platelets did not react after a single dosage of 1000 mg ASA.

b) Influence of 500 mg ASA on PR after two and 12 hours

PR was studied in 61 patients before, two hours and 12 hours after ASA application. Before treatment PR was 1.42 ± 0.46, two hours later 1.04 ± 0.18, and twelve hours later 1.20 ± 0.19. Differences were significant in all cases ($p<0.001$) (Wilcoxon test of paired samples). Six patients of the tested unselected 61 patients showed PR values >1.25 two hours after ASA in ake and so seemed to be primary nonresponder (PNR) while an additional 16 patients were secondary nonresponders (SNR) with index values > 1.25 12 hours later FIG.1.

TABLE 1

Platelet reactivity before and two hours after different orally administered ASA dosages

N.of pat.	platelet reactivity under acetylsalicylic acid											
	1000mg		500mg		200mg		100mg		50mg		M + 100mg	
	bef.	a.2h	bef.	a.2h	bef.	a.2h	bef.	a.2h	bef.	a.2h	bef.	a.2h
1	1.56	1.51	1.6	1.53	1.5	1.41	1.49	1.59	1.61	1.62	1.61	1.01
2	1.44	0.98	1.36	1.00	1.42	1.01	1.45	1.02	1.48	1.00	-	-
3	1.72	0.89	1.67	0.88	1.87	1.00	1.66	0.99	1.81	0.87	-	-
4	1.47	0.99	1.43	0.99	1.38	0.79	1.45	1.41	1.44	1.51	1.42	1.06
5	2.00	1.07	2.11	1.88	1.89	1.99	1.89	1.88	1.86	1.78	1.89	0.96
6	2.11	1.00	1.99	1.01	1.89	0.99	2.00	0.99	2.01	1.78	-	-
7	1.66	1.00	1.59	1.11	1.67	1.01	1.65	1.01	1.61	1.02	-	-
8	1.34	1.11	1.33	1.14	1.30	1.09	1.33	1.17	1.40	1.15	-	-
9	1.41	1.11	1.41	1.18	1.44	1.18	1.44	1.19	1.39	1.20	-	-
10	1.45	0.89	1.35	1.00	1.41	1.01	1.50	1.02	1.42	1.10	-	-
11	1.77	0.99	1.64	0.89	1.70	0.88	1.71	0.93	1.76	1.12	-	-
12	1.29	0.87	1.34	0.89	1.26	0.91	1.28	0.92	1.29	1.00	-	-
13	1.37	0.91	1.27	1.00	1.34	1.00	1.32	1.29	1.31	1.33	1.34	0.89
14	1.55	0.98	1.32	1.19	1.41	1.19	1.51	1.21	1.56	1.56	-	-
15	1.65	0.99	1.55	1.10	1.61	1.11	1.61	1.01	1.64	1.04	-	-
16	1.83	1.07	1.88	1.17	1.82	1.19	1.81	1.12	1.82	1.15	-	-
17	1.81	1.16	1.76	1.13	1.88	1.12	1.88	1.01	1.76	1.00	-	-
18	1.62	1.14	1.55	1.01	1.64	1.02	1.62	0.99	1.63	0.99	-	-
19	1.71	0.99	1.71	0.91	1.69	0.89	1.71	0.98	1.70	1.01	-	-
20	1.48	1.00	1.47	0.99	1.47	0.91	1.48	0.78	1.45	0.88	-	-

pat = patient
M = metoclopramide (10 mg 10 min before ASA-intake)
bef = before ASA application
a.2h= 2 hours after ASA intake

Before onset of treatment, 6% of the PR values, after two hours and 56% and 12 hours after ASA intake 33% of the PR values were found to be within the range of mean + SD of healthy ones (<1.07). After two hours 8 out of all 82 tested ASA treated stroke patients showed PR index values >1.25. 12 hours after ASA intake 30 of 82 patients had index values > 1.25. All patients with pathological two hours PR index values also showed these pathological test values in the measurement 12 hours after ASA intake. These results resembled those found in the subgroup of 61 patients described in FIG.1.

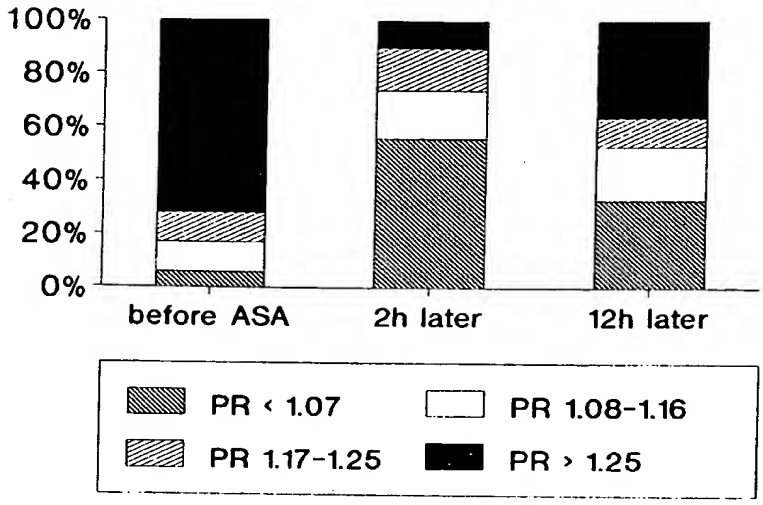

FIG.1 Distribution of platelet reactivity index values of 61 stroke patients under ASA treatment within the normal range of the test system.
before ASA = before 500 mg ASA p.o., 2h later = 2 hours after ASA intake, 12h later = 12 hours after ASA intake. (1.07 = mean + SD (standard deviation) of 110 healthy ones (14). 1.16 = mean +2SD, 1.25 = mean +3SD)

c) Modification of PR with time after ASA intake

Six SNR gave informed consent to bedrest over a period of 12 hours and allowed a blood collection before and during the period of 12 hours every two hours after intake of 500 mg ASA. Before the ASA intake, all patients showed a pathologically increased platelet reactivity, and 12 hours later all patients showed an increased platelet reactivity again. After two hours 100 %, after four hours 84%, after six hours 66%, after 8 hours 50% and after 12 hours, none of these patients showed a normalized platelet reactivity TAB.2. Obviously, once PR index values were increased, they remained pathological, as shown in table 2.

d) Modification of PR after a treatment period of 4 weeks

Ten SNR agreed to be reinvestigated 2 and 12 hours after ASA intake. After a treatment period of 28 days with 3 x 500 mg ASA the laboratory tests were repeated . All patients had initially shown a normal two hours and an increased 12 hours test value. After 28 days of ASA-treatment all patients again showed a normal PR value after two hours and an increased PR value 12 hours later TAB.3.

TABLE 2

Modification of platelet reactivity under ASA in selected secondary nonresponders.

platelet reactivity before	after 500 mg ASA 2h	4h	6h	8h	12h
1.45	0.99	0.98	0.99	1.34	1.40
1.51	1.01	0.99	0.98	0.99	1.55
1.72	1.00	0.99	1.55	1.56	1.49
1.33	0.89	1.21	1.25	1.26	1.29
1.49	0.97	0.91	0.98	1.01	1.42
1.62	0.88	0.99	0.99	1.41	1.44

h = hours after orally intake of 500 mg ASA

TABLE 3

Stability of ASA effects on platelet reactivity in selected secondary ASA nonresponders.

platelet reactivity under 500mg ASA 2.day of treatment		28 days later	
2 h	12 h	2 h	12 h
0.89	1.29	0.89	1.49
0.99	1.35	0.96	1.33
0.98	1.77	1.00	1.60
1.01	1.67	1.05	1.51
1.10	1.66	1.11	1.62
1.11	1.44	1.01	1.41
1.12	1.49	1.02	1.42
1.00	1.57	0.99	1.55
0.89	1.33	0.99	1.29
0.87	1.27	1.00	1.25

h = hours after ASA intake

DISCUSSION:

These findings focus on two different problems concerning ASA treatment in patients suffering from CVD. The first problem is described by the dosage experiment which indicates that about 10% of unselected patients show no ASA induced normalization of PR values two hours after intake of a routinely given dosage of 500 mg ASA . As compared to 500 mg ASA, a dosage of 200 mg seemed have of the same efficacy whereas after 100 mg ASA 20% and after 50 mg even 30% of the tested patients did not react with a normalization of PR. In terms of the laboratory ASA effects two hours after oral ASA intake, an ASA dosage of 500 and 200 mg but not a single dosage of 100 and 50 mg are of comparable effectiveness.

Metoclopramide - a drug that is established as co-drug of ASA in the treatment of acute migraine attacks (17, 18) enabled an additional effectiveness of ASA under these experimental clinical conditions TAB.1. The addition of metoclopramide seemed more effective than the increase of the single ASA dosage to 1000 mg.
The second problem is that the 2 hour follow up experiment TAB.2 suggests that some patients develop an increase of PR in consequence of unknown and unpredictable events. This re-activation of platelets within the 12 hours period is in accordance with observations of BAMBERG et al. (19). FIG.1 suggests that 36% of an unselected group of stroke patients show a PR index value in the absolutely pathological range of the test system (>1.25) only 12 hours after intake of 500 mg ASA. However, 26% of these patients had shown an ASA effect on platelets after two hours. Even a prolonged treatment period does not improve the effectiveness of ASA on platelet function of these SNR TAB.3. So it should be assumed that the "re-activation" of ASA inhibited platelets as shown in the PR test system may be an individual process in these ASA treated patients suffering from CVD. Further experiments are required to characterize these secondary ASA nonresponders, who show such a shortened effect of ASA (below 12 hours) on platelets. Based on other laboratory experiments (8, 13) it is to be assumed that the observed short lasting effects of ASA on the platelet reactivity must be independent of the ASA inhibition of the platelet thromboxane synthesis.
With respect to these findings, the known uncertainity concerning the contradictory results of ASA studies in stroke prevention should be expected - but this is a laboratory point of view that focuses the results of PR testing instead the findings in thromboxane testing. Only a long term follow up of ASA nonresponders and ASA responders may help to clarify whether these laboratory findings are in fact of any clinical relevance.

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